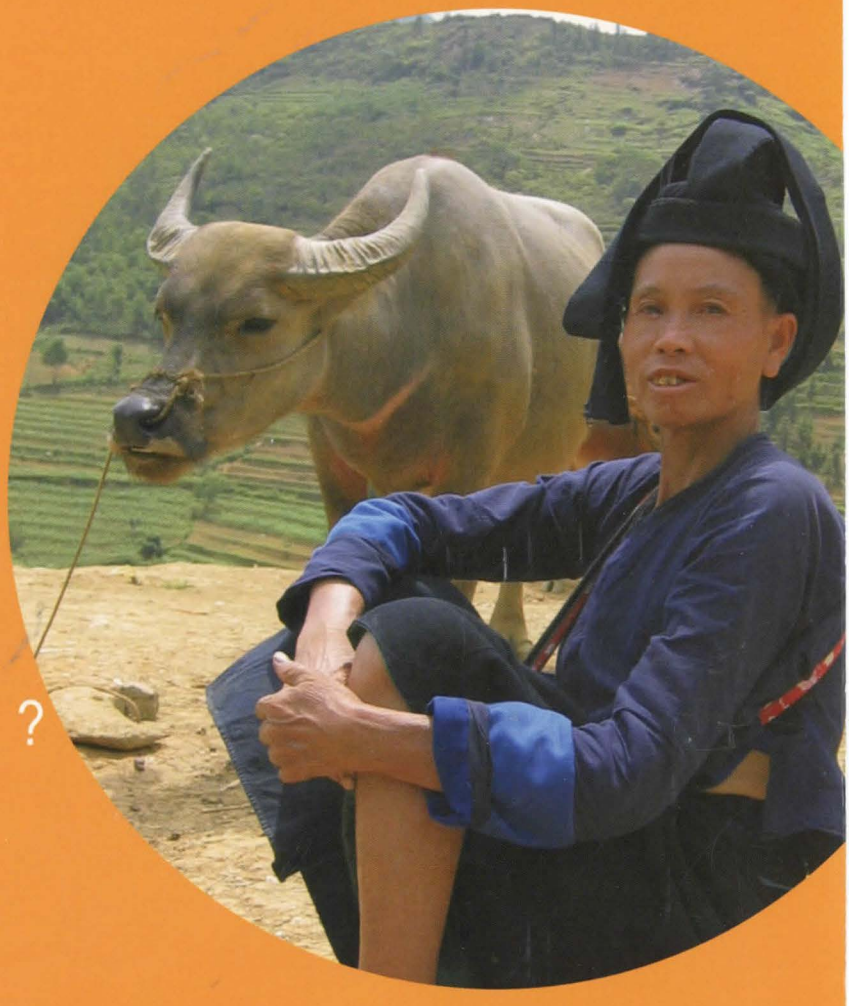
Proceedings

The 12th International Conference of THE ASSOCIATION OF INSTITUTIONS FOR TROPICAL VETERINARY MEDICINE



Does control of animal infectious risks offer a new international perspective ?

Montpellier, France
20-22 August 2007

AITVM

CIRAD

TRYPANOSOMA CONGOLENSE HEAT-SHOCK PROTEIN 70: A PROMISING TH1 ADJUVANT

GARZON E.[1], BOSSARD G.[1], BRAS R.[2], TOTTE P.[1], RODRIGUES V.[1], BOULANGER A.[1], AUTHIE E.[3], DEDIEU L.[1]*

1.Cirad, Control of Emerging and Exotic Animal Diseases Research Unit Campus international de Baillarguet, 34398 Montpellier Cedex 5, France
2.IRD, Unité de pathogénie des trypanosomatidés, 911 Avenue Agropolis, 34394 Montpellier, France
3.INRA, Unité infectiologie Animale et Santé publique, UR1282, 37380 Nouzilly, Tours France

ABSTRACT

Developing immunopotentiators to improve vaccine efficacy in ruminants is an important goal. Heat shock proteins (HSPs) have been shown to act as adjuvant when co-administrated with peptides antigens or given as fusion proteins. In this study, we have investigated the role of *Trypanosoma congolense* HSP-70 (Tcong-HSP-70) on the maturation of murine dendritic cells (DCs) generated from bone marrow precursor cells. The results obtained showed that *Tcong*-HSP-70 induced murine DC maturation as determined by increased levels of surface markers (CD40, CD86 and MHC II) as well as proliferation of allogeneic splenocytes. DCs exposed to *Tcong*-HSP-70 were characterized by strong expression of TNFα and IL-12p70 and moderate expression of IL-10. Similar study, now underway, on bovine monocyte-derived DCs demonstrated the ability of *Tcong*-HSP-70 to also trigger bovine Mo-DC maturation. This finding thus suggests that *Tcong*-HSP-70 can act as a potent Th1 adjuvant for antigen delivery.

INTRODUCTION AND RATIONALE

The control of animal diseases is still a major challenging issue for developed and developing countries. Effective tools for controlling pathologies of major social and economic importance are therefore vital. Among the important components for control are the vaccines. Indeed, in countries where cattle raising rely on nomadism and transhumance, the sanitary measures such as combination of stamping-out, control of cattle movement and quarantine are impracticable.

*Contact author : E-mail: laurence.dedieu@cirad.fr

The trends now in vaccine development focus on sub-unit vaccines as safer and better controlled compared to recombinant viable vaccines. This approach needs the identification of the most efficient protective antigens against each pathology. However, purified proteins are often of low immunogenicity by themselves thus requiring the addition of potent immunostimulating molecules and antigen delivery system. To this aim, our work focused on the identification of new molecules with adjuvant properties.

The effect of these immunostimulating molecules is mainly due to their interaction with the "antigen presenting cells" (APC), the key cells in the immune response. Indeed, these APC, such as dendritic cells and macrophages, are the professional cells capturing any invading pathogen, processing it and initiating the cellular immune response by presenting selected peptides from the pathogen to the T lymphocytes. The adjuvants act by stimulating the APC function, up-regulating several maturation markers (CD40, CD80, CD86…) and the class II molecules of the major histocompatibility complex (MHC) and increasing cytokine secretion, thus allowing enhancement of the resulting cellular immune response.

Our study aimed at characterising the immunostimulating properties of a heat-shock protein 70 isolated from the pathogen *Trypanosoma congolense*. In recent years, heat shock proteins (HSPs) have been shown to interact with APCs and their ability to stimulate both CD8 cytotoxic and CD4 T-lymphocytes has attracted much attention (Pockley, 2001). The HSPs are divided into 6 subfamilies according to their molecular weight including small HSP, HSP40, HSP60, HSP70, HSP90 and HSP110 (Wan et al, 2004). The most intensely studied is the HSP70 family. In their intracellular form, HSP70 function as molecular chaperones for correct protein folding. Instead, in their extracellular form, they have been shown to have a number of immunological effects (binding to APCs, eliciting APC maturation, initiating pro-inflammatory cytokine secretion…).

Our work was thus dedicated to evaluating the function of the HSP70 from *Trypanosoma congolense (Tcong*-HSP70) on APC isolated from mouse and cattle.

RESULTS AND DISCUSSION

A mouse model was used in a first step because of the more difficult handling of cattle and reduced availability of immune cells. The mouse model can thus allow an initial characterisation of the adjuvant

properties, although we are aware of the differences in the immune system of these two animal species.

The results showed that *Tcong*-HSP70 induced the functional maturation of dendritic cells (DCs) and elicited a Th1-promoting response in the mouse model (Garzon et al, submitted). Indeed, the stimulation, by the *Tcong*-HSP70, of murine DCs generated from bone marrow precursor cells, triggered the maturation of the mouse DCs. The results demonstrated increased levels of the maturation markers, CD40 and CD86, as well as of the surface class II molecules of the MHC. Furthermore, the murine DCs exposed to *Tcong*-HSP-70 were characterized by a strong expression of TNFα and IL-12p70, a key mediator of Th1 responses, and a moderate expression of IL-10. Similar results were recently published for HSP70 either from *Trypanosoma cruzi,* the agent of the chagas disease, or from *Toxoplasma gondii* (Planelles *et al.,* 2002; Kang *et al,* 2004).

The study then focused on bovine DCs and macrophages (Mps) using *in vitro* monocytes-derived cells. The results showed a significant difference between both cell types after exposure to *Tcong*-HSP-70 with a stronger response observed on bovine Mps and a moderate one on DCs. Indeed, stimulation of the bovine Mps by *Tcong*-HSP-70 triggered an enhanced level of expression of the maturation markers CD40 and CD80 and a dominant IL-12 secretion over the IL-10 production. Instead, although DC exposure led to an increase of the CD40 expression, the level was less than half the one observed for the Mps. Similarly, the level of Il-12 produced by the *Tcong*-HSP-70-stimulated DCs was 5 times lower than detected for the Mps.

Therefore, the HSP70 from *Trypanosoma congolense* has the ability, in the mouse model, to trigger DC activation with a Th1 dominant cytokine profile. In contrast, in the bovine system, *Tcong*-HSP-70 elicits only a moderate DC maturation while a stronger effect was detected on the Mps with also a Th1 dominant cytokine profile. Consequently, *Tcong*-HSP-70 is able to modulate the host immune system, leading to an orientation towards a TH1-biased response.

CONCLUSION

The protein HSP70 from *Trypanosoma congolense* might thus be used in new antigen delivery systems, as a promising Th1 adjuvant, in order to orientate and enhance a Th1 host immune response. This type of immune

response plays a major role in the protective immune response against several bovine pathogens such as *Mycoplasma mycoides* subsp. *mycoides* S.C., the agent of the contagious bovine pleuropneumonia or *Mycobacterium bovis*, the agent of bovine tuberculosis. Further work is now dedicated to 1) the identification of the protein fraction responsible for this adjuvant property and 2) to confirm the immunostimulating role of the *Tcong*-HSP-70 on antigenic molecules.

REFERENCES

Garzón E., Bossard G., Bras R., Totté P., Rodrigues V., Boulanger A., Autié E., Dedieu L. *Tcong*-BiP, a heat-shock protein 70 from *Trypanosoma congolense,* induces functional maturation of dendritic cells towards a Th1-promoting phenotype. Submitted to Developmental and Comparative Immunology.

Kang H.K., Lee H.Y., Lee Y.N., *et al,*. 2004. *Toxoplasma gondii*-derived heat shock protein 70 stimulates the maturation of human monocyte-derived dendritic cells. Biochem Biophys Res Commun, 322: 899-904.

Planelles L., Thomas M., Pulgar M., Maranon C., Grabbe S., Lopez MC., 2002. *Trypanosoma cruzi* heat-shock protein-70 kDa,alone or fused to the parasite KMP11 antigen, induces functional maturation of murine dendritic cells. Immunol Cell Biol., 80: 241-7.

Pockley A.G., 2001. Heat shock proteins in health and disease: therapeutic targets or therapeutic agents. Expert Rev Mol Med. 2001: 1-21.

Wan T., Zhou X., Chen G., *et al.*, 2004. Novel heat shock protein Hsp70L1 activates dendritic cells and acts as a Th1 polarizing adjuvant. Blood. 103: 1747-54.